INTRACEREBROVENTRICULAR INJECTION OF INTERLEUKIN 1 INDUCES HIGH CIRCULATING LEVELS OF INTERLEUKIN 6

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There is a growing interest for connections between the central nervous system (CNS) and systemic immune and/or inflammatory responses. The inflammatory cytokine IL-1 has a wide spectrum of targets, which include the CNS. Intracerebroventricular as well as systemic administration of IL-1 were reported to induce central effects including fever (1), slow-wave sleep (2), anorexia (3), and activation of the hypothalamus-pituitary axis leading to release of adrenal corticosteroids (4, 5). Moreover, different brain cells such as astrocytes, microglia, and neurons can respond and/or have been found to contain various cytokines like IL-1, IL-2, IL-6, and TNF, and there is evidence that these factors act on neuronal survival, growth, and differentiation (1, 3, 6-10).

It is also possible that some of the systemic activities of IL-1 are, at least in part, centrally mediated. In fact, early reports indicated that central administration of crude leukocytic endogenous mediators (presumably containing, among other cytokines, IL-1) induced an increase of acute-phase proteins (11). The mechanism by which centrally administered IL-1 can activate the synthesis of hepatic acute-phase proteins is still unknown, and the present study was aimed at revisiting this early observation by investigating how intracerebroventricularly administered rIL-1 induced a systemic response. Our attention was focused on IL-6, since this cytokine, induced by IL-1, plays a crucial role in the acute-phase response as a hepatocyte-stimulating factor, and the levels of circulating IL-6 were reported to correlate with the levels of acute-phase proteins in some infective and inflammatory diseases (12-14).

Materials and Methods

Male rats (250-300 g) (CD-COBS, Charles River Breeding Laboratories, Inc., Calco, Como, Italy) were used. They were housed with free access to food and water, under a 12-h light/dark cycle with constant temperature (21-23°C) and humidity (20-25%).

IL-1 (human rIL-1-β; a kind gift of Sclavo, Siena, Italy) was injected either intraperitoneally, intravenously, or intracerebroventricularly through one polyethylene cannula permanently implanted in the lateral ventricle 3 d before the experiment (15). IL-1 was dissolved in sterile,
pyrogen-free saline containing 0.1% BSA and administered at the dose of 200 ng/5 μl for each rat. Control rats were given the same volume of vehicle.

Indomethacin (Chiesi Farmaceutici, Parma, Italy) was administered at the dose of 20 mg/kg, i.p., 1 h before IL-1.

10 d before the experiment, one group of rats was hypophysectomized according to the procedure described by Falconi and Rossi (16); another group was adrenalectomized and given 1% NaCl dissolved in drinking water. For each experiment, appropriate sham-operated rats were used as controls.

Rats were killed by decapitation, blood was collected, and serum was prepared. IL-6 in serum samples was measured as hybridoma growth factor using the 7TD1 cell line obtained through the courtesy of Dr. J. Van Snick, Brussels, Belgium, as previously described (17). 1 U in the 7TD1 assay corresponded to 1 pg human rIL-6. The sensitivity of the assay with rat serum was 50 U/ml.

Results and Discussion

Fig. 1 shows the effects of intracerebroventricular administration of IL-1 (200 ng/rat) on serum IL-6 levels at 2 h after IL-1. The results of two different experiments are reported, where the effect of intracerebroventricular administration was compared with that of systemic administration (intraperitoneal or intravenous) of the same amount of IL-1. It is clear that centrally administered IL-1 induced markedly higher levels of IL-6 than systemically given IL-1. The time course of IL-6 induction by IL-1 given intravenously or intracerebroventricularly was comparable, as shown in Fig. 2, with a peak at 2 h. When heat-inactivated (90°C, 20 min) IL-1 was injected intracerebroventricularly, no IL-6 induction was observed (IL-6 levels were <50 U/ml), thus ruling out the possibility that endotoxin contamination of the rIL-1 preparation could be responsible for the observed effect.

The higher serum IL-6 levels observed when IL-1 was administered intracerebroventricularly rather than systemically clearly rule out the possibility that induction of
circulating IL-6 by intracerebroventricularly administered IL-1 might be due to a passage of IL-1 into the circulation through the blood brain barrier.

The effect of IL-1 on hypothalamic thermoregulatory centers is known to be mediated by prostaglandins (1, 18). It was therefore important to evaluate whether products of arachidonate metabolism were involved in induction of systemic IL-6 by intracerebroventricular IL-1. We have studied the effect of pretreatment with an inhibitor of prostaglandin synthesis, indomethacin (20 mg/kg, i.p., 1 h before IL-1), which was previously shown to inhibit the pyrogenic action of IL-1 administered intracerebroventricularly (19, 20). The results reported in Fig. 3 show that indomethacin did not abolish the effect of centrally administered IL-1 on serum IL-6 levels. It should be noted that indomethacin alone increased serum IL-6 levels, although to a lesser extent than IL-1. Maximal induction of IL-6 by indomethacin alone was at 2 h, and was also observed with 30 mg/kg of another cyclooxygenase inhibitor, ibuprofen (data not shown). One possibility for the inducing effect of cyclooxygenase inhibitors on IL-6 levels is that prostaglandins provide an inhibitory signal for IL-6 synthesis, as it was reported for IL-1 (21).

IL-1 activates the hypothalamus-pituitary axis, causing release of pituitary hormones and, ultimately, glucocorticoids (4, 5). It was therefore important to ascertain whether the activation of the hypothalamus-pituitary axis is responsible for the induction of serum IL-6 by centrally administered IL-1. For this purpose, we have studied the induction of IL-6 in hypophysectomized rats. As shown in Fig. 4 A, hypophysectomy did not block the induction of IL-6 by centrally administered IL-1. Indeed, in all the experiments, hypophysectomy increased the IL-6 response.

We have also considered the possibility that IL-6 could be released by the adrenals, which were reported to contain high levels of IL-1 that could be released by degranulation of catecholaminergic terminals (22). Results obtained using adrenalectomized rats are shown in Fig. 4 B. Adrenalectomy also increased the induction of IL-6 by centrally administered IL-1. It should be pointed out that adrenalectomy, like hypophysectomy, increased IL-6 levels even after intraperitoneally administered IL-1 (data not shown), confirming our previous reports of a higher sensitivity of adrenalectomized animals to IL-1, probably due to the absence of feedback mechanisms mediated by corticosteroids (23).

Taken together, these data rule out the possibility that induction of serum IL-6

Figure 3. Effect of indomethacin (INDO) pretreatment on IL-6 induction by intracerebroventricularly administered IL-1 (200 ng/rat). Data represent mean ± SD of four to five rats per group.
by centrally administered IL-1 is secondary to its pyrogenic action mediated by prostaglandins or to the stimulation of the hypothalamus-pituitary axis.

The finding that intracerebroventricular administration of IL-1 induces circulating IL-6 levels extends the list of central activities of IL-1 and indicates the existence of a novel pathway that could explain how infections or lesions confined to the CNS result in systemic alterations of acute-phase response parameters. The role played by IL-1 under conditions involving disturbances in the neurotransmission has been up to now poorly investigated. Glia cells are known to synthesize and store IL-1, as well as other cytokines (6-10), and reactive gliosis with elevated IL-1 activity has been recently reported after brain injury and in neuropathological diseases like Down's syndrome and Alzheimer's disease (24, 25). It was also shown that elevated levels of IL-6 are present in the cerebrospinal fluid of patients with meningitis (26), and both IL-1 and IL-6 were found in cerebrospinal fluid of patients with HIV-1 infection of the CNS (27).

The origin of the high blood levels of IL-6 induced by intracerebroventricular IL-1 remains to be established. IL-6 could be produced in the brain, for instance, by microglial cells (8, 10) or endothelial cells in the plexus chorioideus; alternatively, via an yet undefined pathway, production could be induced at peripheral sites.

The mechanism by which IL-1 can stimulate IL-6 production by a CNS-mediated pathway is still unclear. Studies are in progress to investigate the brain areas and the neurotransmitters implicated in this effect of IL-1 on the CNS and the source.
of the IL-6 released. Whatever the cellular origin and pathways involved, the observations reported herein provide a link whereby IL-1 produced intracerebrally or reaching the CNS can elicit a systemic acute-phase response.

Summary

IL-1 is known to have a central role in the induction of acute-phase response, and some of its activities (including induction of some acute-phase proteins) were reported to be mediated by an induction of IL-6. Administration to rats of 200 ng of human rIL-1 by intracerebroventricular injection resulted in a more marked induction of circulating IL-6 than the same dose of IL-1 administered systemically (intravenously or intraperitoneally). Induction of serum IL-6 by centrally administered IL-1 was also observed in hypophysectomized or adrenalectomized rats, suggesting that activation of the hypothalamus-pituitary-adrenal axis is not essential for this effect of IL-1. IL-6 induction was also observed after pretreatment with indomethacin, indicating that the effect was dissociated from the pyrogenic activity of IL-1. Induction of IL-6 by a central action could represent a novel pathway in IL-1-induced acute-phase response.

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References


